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BACTERIAL LEAFSPOT OF GERANIUM IN THE EASTERN UNITED STATES[1]

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The florist's season for growing cuttings of geraniums (*Pelargonium* spp.) for garden and window-box planting is the season when this bacterial disease may be looked for, especially in crowded, ill-ventilated houses. Nearly every spring since 1912 and at any time during March, April, and May, according to the locality where the plants are grown, we have received diseased specimens. Very rarely has a diseased plant grown in the open been sent to us. Our first acquaintance with it, however, was at an earlier date when it occurred on the grounds of the United States Department of Agriculture in Washington (Pl. 1, A). In New Jersey and Maryland the disease has been especially prevalent and of a destructive nature, whole houses of rooted plants in some instances becoming unsuitable for market. It is a spot disease which makes the leaves unsightly and undermines the health and development of the cuttings. The year 1915 was evidently favorable for the disease, for it occurred very generally in the eastern part of the United States.

The spots are definitely outlined, usually irregular but occasionally circular in shape, and of a brown color. The spotting may begin either at the margin of the leaves or on the blade and may occur on either old or young leaves, although the older leaves or those nearest the soil are usually the worst affected. The youngest infected areas are watery looking, then become a reddish brown, later the tissue dries and becomes dark brown. When the disease is advanced the portion of the leaf between the spots turns dark and becomes dry and wrinkled. The spots, however, still show plainly in the dead area. The bacteria, which are motile, occur in great numbers in the spots. They are easily isolated from the young spots, as the epidermis can be sterilized with mercuric chlorid (1 to 1,000) for three to five minutes without much penetration.

Isolations were made from material received from Maryland (Pl. 1, B), and water suspensions of subcultures were sprayed on young geranium plants growing in the greenhouse. The plants were kept in a moist infection cage for two days. In another cage, which was also kept moist, were other geranium plants of the same age which were sprayed with sterile water. Typical spots appeared on the inoculated (sprayed) leaves in 9 days, and in 17 days the plants were badly spotted (Pl. 3, B). The controls did not show any spotting. Bacteria were abundant in the spots. The organism was reisolated and other geranium plants were infected with it by spraying. In four days spots began to show on the leaves of these sprayed plants. The temperature was higher at this time, being 80° F. during most of the daytime.

[1] Accepted for publication May 10, 1922.

Likewise, isolations were made from diseased material from New Jersey (Pl. 2). The same organism was obtained and typical spots produced in 17 days from the time of spraying the leaves with a water suspension of an agar subculture (Pl. 3, A). The organism was reisolated also from this New Jersey strain. The inoculations with the reisolated New Jersey organism were made in two different greenhouses, one in which the day temperature was 70° to 75° F. and another in which it was 55° to 60° F. The plants were sprayed and kept in infection cages for two days. In the warmer house infection occurred early and was well marked in 16 days, while there was but a trace of infection on the inoculated leaves in the cooler house. The disease did not progress in the latter case but continued in the former.

A bacterial disease of geranium plants was reported from Massachusetts [2] in 1898. The observers first found it on several different varieties during a season of rainy weather about the latter part of July on plants grown out of doors. The leaves were spotted and bacteria were found in the spots.

Another report of the disease from Massachusetts [3] stated that attempts had been made to isolate the organism but without success.

Still another report [4] said the disease had been noticed in that State every year for nine years. It was abundant and generally distributed, and gardeners had become concerned about it. The spotting was not serious in greenhouses, however, and it was thought to have been brought in from out-of-door stock.

Dr. Erwin F. Smith,[5] in volume 1 of his "Bacteria in Relation to Plant Diseases," mentioned the disease as one produced by stomatal infection and in volume 2 called it a disease of rainy seasons. In volume 2 he also stated that the organism was isolated in his laboratory and the disease reproduced on geranium leaves by John R. Johnston, inoculating from pure cultures. No further work on this disease was done by Mr. Johnston or by Dr. Smith beyond recognizing it as a yellow organism with a polar flagellum. He states to me that none of the earlier isolations from the Washington material ever greened the medium.

A bacterial disease of the leaves of Erodium and Pelargonium in Texas was described in Phytopathology [6] for August, 1914. The general appearance of the spots on the leaves in the illustrations of the article led us at first to think that the Texas disease described was the same as the one our laboratory had been dealing with, although Lewis's figures 2 and 3 indicate a much more active parasite. When comparisons of cultural tests were made and what seemed to be important differences were noted, it was decided that the two organisms must be different and that these different features would be accentuated if worked out in more detail. That has been done, and careful observation and repeated tests have established the writer's belief that the organisms are not the same, for in cultural tests important differences persist. Perhaps Mr. Lewis's work was done with the Erodium strain and the host may be responsible for these differences. He does not state in his paper which isolation he

[2] Stone, George E., and Smith, Ralph E. A disease of the cultivated geranium. *In* Mass. Agr. Exp. Sta. 10th Ann. Rpt., 1897, p. 67, 1 pl. 1898.

[3] ———— A geranium disease. *In* Mass. Agr. Exp. Sta. 12th Ann. Rpt., 1899, p. 57–58. 1900.

[4] Stone, G. E., and Monahan, N. F. Bacteriosis of geraniums. *In* Mass. Exp. Sta. 19th Ann. Rpt., p. 164. 1907.

[5] Smith, Erwin F. Bacteria in relation to plant diseases. v. 1, p. 92, 1905; v. 2, p. 39, 62, 1911. Washington, D. C. Carnegie Inst. Publ. 27.

[6] Lewis, I. M. A bacterial disease of erodium and pelargonium. *In* Phytopathology, v. 4, no. 4, p. 221–232, pl. 10. 1914.

used for his cultural tests. He states that he isolated an organism from both Erodium and Pelargonium and that he was convinced that the organism from the wild Erodium and the cultivated species of geranium (Pelargonium) were one and the same. He found that both strains cross-inoculated readily but does not mention making any comparative cultural tests of the two strains. In all probability he used one strain only for his cultural tests.

The comparisons in this paper were started with the idea of finding enough agreement in the cultural tests to establish the identity of our organism as *Bacterium erodii*, and with that in view we cast about for explanations to account for the differences. One striking difference between the Texas and Maryland organisms is in the production by the Texas organism of a green fluorescence in beef agar, beef bouillon, sterile milk, and various other media. Very careful observations were made, but no trace of green fluorescence could be detected at any time in the tests with the Maryland or New Jersey isolations. The continued comparisons in this paper were made with the New Jersey isolation. Morphologically the Texas and New Jersey organisms are much alike and in some of the cultural tests are identical. Although in two to five days we have never obtained leaf-spots such as Lewis figures the two organisms produce somewhat the same type of disease on geranium leaves apparently under the same conditions, yet if they are the same organism why does one produce green pigment and the other not?

Thinking the green color might have formed through some particular property of the medium, two lots of peptone-beef bouillon (beef infusion) were made in which Witte's and Difco peptone were used as well as two lots of beef extract media with the two kinds of peptone. Our organism acted the same in all in respect to color—there was not a trace of greening. Then Mr. Lewis's platings from diseased material were considered. At the start an organism appeared on his plates which greened the agar. Subcultures from these colonies picked from the plate produced the disease. Could this pigment formation be the individuality of a strain? If so, it is a striking variation and a feature that must be reckoned with when comparing strains and varieties in proving up a new organism.

The New Jersey strain of the geranium leafspot disease was used for the tests described hereafter in this paper. To facilitate comparison with *Bacterium erodii* the same order is followed here as in the article by Lewis describing that organism.[7] No work was done with *Bacterium erodii* itself, as we had no culture of that organism nor were we able to obtain Texas material for our own isolations. The comparisons, therefore, were all made with the rather full tests published by Mr. Lewis in the article just cited.

CULTURAL CHARACTERISTICS OF THE ORGANISM FROM THE DISTRICT OF COLUMBIA, MARYLAND, AND NEW JERSEY

AGAR PLATES.—On beef infusion peptone agar +16.5 plating from a 2-day-old bouillon culture, colonies do not appear until the third day. In reflected light they are cream color, shining, round with a smooth surface. In transmitted light under a hand lens they are a cream color in the center and bluish outside of center. There are delicate reticulate

[7] LEWIS, I. M. OP. CIT.

markings in the interior; there is no zoning, but a marginal ring occurs on some colonies after they are up several days. Colonies are 2 to 4 mm. in diameter. The agar does not change color. (Unlike the Lewis organism.)

AGAR STROKE.—In 2 days there is a translucent wet shining rather thin growth on +13 peptone-beef infusion agar. In 3 days the growth is cream colored with undulating surface. There is no green color in the agar or condensation water. The surface is finely pitted at 4 days; crystals form in from 2 to 10 days. There is no viscidity until cultures are 1 month old or older, but even then there is no green color. (The Lewis organism produces a green pigment.)

GLUCOSE AGAR STAB.—This is a favorable medium. Growth is fairly rapid and abundant on the surface of the stab; scant along the line of puncture. The color of 4-day-old cultures is Ridgway's Naples yellow,[8] and this is a deeper color than the growth on plain beef agar. (Same as the Lewis organism.)

LACTOSE AGAR STAB.—In this medium there is growth with abundant crystals. No green color. (The Lewis organism produces a green color.)

STEAMED POTATO CYLINDERS.—A thin yellow growth occurs on the potato in 24 hours. In 2 days it is a Naples yellow; in 3 days the potato begins to darken a little, but there is no green fluorescence. The potato cylinders are still firm after a month. The starch in the cultures gives a purple reaction when tested with iodin in potassium iodid. (Lewis's organism produces a green fluorescent pigment after 48 hours.)

STEAMED COCONUT.—A thin faintly yellow growth occurs in 2 days on pieces of coconut steamed in tubes. Growth takes place in the water also but is not a yellow color. In 2 weeks the growth on the surface of the coconut is still thin and not viscid. (The Lewis organism produces a viscid growth on this medium.)

LITMUS MILK.—There is a trace of clearing (whey) in 3 days but no color change. In 6 days there is a faint bluing in bands and a clearing at the surface for nearly 1 cm., but no coagulation. In from 10 to 15 days the bands of color are faint and clearing has taken place in from one-half to the entire tube. Then it is a reddish blue color, dark hyssop violet, according to Ridgway. (With Lewis's organism the liquid is all a clear yellowish color in 8 days.)

STERILE MILK.—No clearing occurs before 5 days even at the optimum temperature of 26° to 28° C. At that time there is a ring of whey, cream colored, 3 to 5 mm. deep. Coagulation comes soon after the clearing appears; the curd is soft. There is no color change. Tested at 17 days the hydrogen-ion concentration expressed in P_H value is 6.4 (Brom cresol purple). In 30 days over half the curd has been digested. (The Lewis organism does not coagulate sterile milk; it produces green fluorescence with age.)

DUNHAM'S SOLUTION.—There is heavy clouding in this medium in 48 hours, but not any marked difference from peptone bouillon. (Like the Lewis organism.)

PEPTONE BOUILLON.—The organism clouds peptone beef infusion bouillon +10 to +15 in 24 hours at a temperature of 23° to 27° C., when transfers are made from a young fluid culture. At 18° to 20° it is not clouded until 48 hours. Clouding is heavy in 2 days at 27°. Pseudo-

[8] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

zooglea are present. In 2 to 4 days an incomplete pellicle is formed and the medium is clear below the pellicle. Cultures are not viscid until 1 month old or older. When the tube is agitated the pellicle drops to the bottom in flocks. No green color is produced in either young or old cultures. Some bouillon was made containing Witte's peptone, and the organism grown in it was compared with that containing Difco peptone to see whether the green color would appear. It did not, nor when extract of beef was used with the two kinds of peptone instead of the beef stock. There was no trace of a green color at any time. (The Lewis organism greens the bouillon.)

DUNHAM'S SOLUTION WITH METHYLENE BLUE.—The organism grows well in Dunham's solution colored with methylene blue. The color changes to a faint blue in four days. This test was repeated with the same result. The color reduced from the bottom of the tube upward. In 22 days the methylene blue is reduced to a mere trace. In 1 month after inoculating the color begins to return, but is a green instead of blue—light hellebore green, according to Ridgway.[9] (The Lewis organism does not change the blue color; observations were made over a period of 4 weeks.)

BOUILLON WITH SODIUM CHLORID.—Growth takes place in neutral beef bouillon to which 2.5 per cent sodium chlorid is added. Tests were made with 3.5 and 4 per cent sodium chlorid, but no growth took place in either of the latter media. (Like the Lewis organism.)

BOUILLON OVER CHLOROFORM.—No growth occurs in 10 cc. of peptone-beef bouillon over 5 cc. of chloroform. Three different tests were made, one in which the quantity of chloroform was reduced to 3 cc. No growth occurred. The same bouillon inoculated without chloroform showed abundant growth in 48 hours. (The Lewis organism grows in this medium.)

DECOCTION OF GERANIUM LEAVES.—Fifty gm. of fresh geranium leaves were boiled in a liter of water, then filtered and autoclaved. It tested +6, Fuller's scale; P_H 4.1. The organism was slow in appearing and grew but feebly in this medium. (Not unlike the Lewis organism.)

GERANIUM AGAR.—Some of the decoction described above was made up into agar by adding 2 per cent agar. The medium titrated +6 Fuller's scale; P_H 4.1. A mere trace of growth occurred in the agar made from the decoction. (Not unlike the Lewis organism.)

NITRATE BOUILLON.—There is slight growth in 2 days in beef bouillon containing 1 per cent potassium nitrate; good growth in four days. A partial pellicle is formed rather heavy with crystals. This pellicle falls apart in handling the tube. (The Lewis organism makes a persistent pellicle.)

FERMI'S SOLUTION.—In 2 days there is a faint clouding at temperatures of 25° to 28° C. The clouding is still faint in 2 weeks. In older cultures an incomplete pellicle is formed. There is no green fluorescence with age. A culture 3 months old is Ridgway's old gold color. (The Lewis organism produces green fluorescence.)

COHN'S SOLUTION.—There is no growth in Cohn's solution. (The Lewis organism grows in Cohn's solution without greening.)

USCHINSKY'S SOLUTION.—At temperatures 25° to 28° C., which are favorable temperatures for this organism, there is only a mere trace of growth in 2 days, and no heavier at 7 days. In 2 weeks clouding is better,

[9] RIDGWAY, Robert, OP. CIT.

and later an incomplete pellicle forms. There is no change in the color of the medium when cultures are 3 months old. (The Lewis organism grows promptly in this medium without greening.)

GELATIN STAB.—With +10 beef-infusion gelatin, stab and plates, the liquefaction occurs slowly. Plates thickly sown show slight liquefaction in 7 days, and most of the plate liquefies in 12 days. Temperature 18.5° to 20° C. The liquefaction starts in the stab cultures in 6 days and continues slowly across the surface. In 1 month they are about one-fourth liquefied. In 2½ months the stabs are slightly over half liquefied. In 3½ months one stab was entirely liquefied and the others three-fourths. In 4½ months all cultures are entirely liquefied. (The Lewis organism liquefies a stab culture in 4 weeks.)

LACTOSE LITMUS AGAR.—Growth takes place readily. There is no color change in 5 days. In 7 days the bacteria have taken up color, and a mass on a platinum loop looks green. The slant has become blue, but the agar at the bottom of the tube has not changed color. In 16 days the color of slant is still blue; that part of the medium in the bottom of the tube is unchanged. (The Lewis organism reddens the medium.)

STEAMED CARROT CYLINDERS.—Growth does not take place quickly on carrot cylinders, but in 12 days the surface of each is covered with a creamy growth, smooth, wet shining, not viscid. There is no browning of the medium in 25 days. (The Lewis organism browns the medium.)

STEAMED WHITE TURNIP.—Growth is slow in starting on this medium and is always thin. It is creamy in color, wet shining, not viscid. The medium is neither softened nor browned in 25 days. (Mr. Lewis says this medium is favorable for growth and that it becomes soft and brown.)

INDOL.—There is a slight production of indol in Dunham's solution cultures 10 days old. It is still slight when the cultures are 18 days old. The tests were made with sulphuric acid and sodium nitrite. (Like the Lewis organism.)

HYDROGEN SULPHID.—Hydrogen sulphid is produced. The organism was grown on potato cylinders, beef agar, lactose agar, and in beef bouillon. The test was made by suspending lead acetate paper in the culture tubes. The paper became well blackened in every case. (The Lewis organism does not produce hydrogen sulphid in any of these media, not even after prolonged exposure.)

AMMONIA PRODUCTION.—The organism produces ammonia. Cultures of beef bouillon and peptone water both 10 days and 3 weeks old were tested with Nessler's solution. Strips of filter paper were moistened with the solution and suspended in the tubes to be tested. The cultures were then heated in a water bath. A red-brown color appeared on the filter paper immediately. (Same as Lewis's organism.)

NITRATE REDUCTION.—There is no reduction of nitrates to nitrites. Tests were made with nitrate bouillon in which the organism grew very well. Ten-day and 28-day cultures were tested. *Bacillus coli* grown in the same medium and tested by the same method (starch-iodin sulphuric acid test) gave a positive test. (Same as Lewis's organism.)

REDUCTION OF LITMUS.—Litmus is reduced in 10 to 15 days in sterile milk. (Not appreciably different from Lewis's organism.)

METHYLENE BLUE.—Reduction of methylene blue takes place in 7 to 11 days, according to temperature. Tests for the reduction were made in milk colored to a robin's egg blue. The reduction begins from the bottom of the tube and goes upward. In 3 days it is white at the bottom of the tube for one-eighth of the liquid. The rest of the liquid is a slightly lighter blue than the control. No coagulation occurs. In 11 days there is entire reduction in some tubes. When entirely reduced the milk coagulates. Those tubes with a slight blue at the surface are not coagulated. In 15 days the blue in all tubes is wholly reduced. Temperature 20° to 22° C. When tubes of methylene blue milk are inoculated and placed at 27° to 28° reduction occurs throughout in 6 days. (With the Lewis organism the tests for the reduction of methylene blue were negative.)

THERMAL RELATIONS

THERMAL DEATH POINT.—The thermal death point lies between 51° and 51.5° C. Transfers were made from well-clouded 24-hour-old cultures and tested many times. The beef bouillon titrating +13 to +17 on Fuller's scale (P_H 6.8 to 7.0) was held in thin-walled test tubes and after transfers were made was kept and heated for 10 minutes at constant temperature in a water bath. Growth occurred at all temperatures tried (48°, 48.5°, 49°, 49.5°, 50°, 50.5°, 51°) except 51.5°. Once in six tubes there was no growth at 50°, but in another six tubes all grew at 50.5°. In one out of two tests growth took place at 51°. None at 51.5°. (The thermal death point of the Lewis organism is 48.5°.)

OPTIMUM TEMPERATURE.—The optimum temperature is around 27° C. Tests were made with temperatures from 10° to 40°. The organism clouds +15 bouillon in 24 hours from 23° to 28°, but in 48 hours the growth is heavier at 26° to 28°. (Agrees with the Lewis organism.)

MINIMUM TEMPERATURE.—Growth occurs at 1° C. In one test with the organism grown in +15 beef bouillon there was a trace of clouding in 16 days. The temperature ranged from −1° to 1.75°. In a second test in which the thermostat ranged from 0.6° to 1.25°—never over 1.25° and usually under 1°—growth was visible in 49 days. It was never more than a faint clouding. (The Lewis organism grows in both beef agar and beef bouillon at 0°.)

MAXIMUM TEMPERATURE.—Growth occurs at 35° C, but it is very feeble. There is none at all at 36° to 37°. (Very little difference from the Lewis organism.)

TEST FOR ANAEROBISM

The organism will not grow in an atmosphere deprived of oxygen. Agar and bouillon transfers were placed in a specially devised jar from which the oxygen was removed as follows: A solution of 35 gm. of potassium hydroxid dissolved in 350 cc. of water was poured over 40 gm. of pyrogallic acid. The bottle for this solution had previously been adjusted in a jar where the transfers were. The mixture was poured in the bottle and its top left uncovered; but the jar was covered, then another cover inserted in a bed of mercury was placed over the whole. A tube of methylene blue milk and litmus milk had been placed in the jar with the cultures as controls on the presence of oxygen.

No growth occurred in any of the cultures in the jar. The bouillon control in the room showed good growth. The methylene blue had faded considerably in 11 days. The litmus did not fade until 15 days after setting up the experiment. A careful watch was kept for over 3 weeks, but no growth took place in the cultures until they were removed from the jar. (Like the Lewis organism.)

RELATION TO MOISTURE

This test was followed out according to Mr. Lewis's rod method. Glass rods were held in place in test tubes by passing them through the cotton plugs, after which the tubes were sterilized. Next the rods were dipped to a uniform depth in a 6-day-old bouillon culture, returned to the tubes, and left to dry at room temperature which varied from 18° to 23° C. Care was taken that the rod did not rest against the side of the tube and prevent a uniform drying. At intervals of 24 hours several of these rods were transferred each to a tube of beef bouillon. Growth occurred in one test after the cultures on rods had been dried 7 days. In a second test made exactly the same way, growth did not occur when dried 7 days but did occur at 6 days. Evidently 6 days is about the limit of drying for a 6-day-old culture of this organism. (Like Lewis's organism.)

GROWTH IN FERMENTATION TUBES

GAS FORMATION.—The organism is aerobic so far as tested and does not form gas. It was tested in fermentation tubes in the presence of each of the following carbon compounds: saccharose, dextrose, lactose, maltose, and glycerin; 1 per cent of these being added to a 2 per cent water solution of Witte's peptone. No gas formed in any of the tubes. Growth occurred in the open arm of each tube but none in the closed arm.

The cultures were tested for acidity after they had grown 21 days. Five tests were made—two in January, in which Witte's peptone was used in one and Difco peptone was used in the other. Another test containing Witte's peptone was made in June. The fourth and fifth tests were made in July with Witte's and Difco peptone, as shown by Table I. Tests 1, 2, and 3 are with Witte's peptone, 4 and 5 with Difco. The results are as indicated in Table I, and it is interesting to note how the same organism varies. With Difco peptone, the acidity of the medium was considerably reduced by the growing organism.

Titrations with phenolphthalein were made before inoculating and again after the organism had grown 21 days. The first test after 21 days showed there was little change in acidity in the cultures. The saccharose, dextrose, and glycerin cultures were slightly more acid than the controls; the maltose and lactose cultures were less acid. In the second test after 21 days all cultures were more acid than the controls. In the third test after 21 days there was no change in acidity with dextrose, lactose, and glycerin; there was increase with saccharose and decrease with maltose. The fourth and fifth tests after 21 days were made with Difco peptone. Titrations showed there was decidely less acidity in the cultures than in the uninoculated media.

To determine further the changes in acidity, some of each of the same medium was tested with the indicators brom thymol blue, brom cresol purple, phenol red or cresol red, before inoculating. This was done at the same time they were tested on Fuller's scale. In all five tests the

P_H values were found to vary from P_H 7.0 to 6.6—that is, from neutrality slightly to the acid side. After inoculating, vigorous growth occurred in all cultures. At the end of 21 days tests were again made with the indicators. There was no increase in the hydrogen-ion concentration. As indicated by the records (P_H 7.2 to 8.6) there was decrease in the hydrogen-ion concentration. In all five tests there was a uniformity of these changes in acidity, as shown by the indicators named above.

The titrations with phenolphthalein of cultures grown in carbohydrate media containing Witte's peptone were somewhat different from those made by Mr. Lewis with his organism, though not so strikingly so as with the Difco peptone. The second test was the only one that showed an increase in acidity throughout.

At the time of Mr. Lewis's experiments (his work was published in 1914) Witte's peptone was in general use in laboratory media.

TABLE I.—*Acidity of cultures of the writer's geranium leafspot organism*

Medium.	P_H.					Fuller's scale.				
	Witte's peptone.			Difco peptone.		Witte's peptone.			Difco peptone.	
	First test.	Second test.	Third test.	Fourth test.	Fifth test.	First test.	Second test.	Third test.	Fourth test.	Fifth test.
Saccharose:										
Uninoculated.....	7.0	7.0	7.0	7.0	6.8	+8.5	+9	+10	+10	+13
Inoculated 21 days..	7.6	7.6	7.2	8.2	7.2	+12	+17	+15	0	+11
Dextrose:										
Uninoculated.....	6.8	6.7	6.8	6.8	6.6	+8.0	+10	+10	+11	+12
Inoculated 21 days..	7.6	7.8	7.4	7.4	7.4	+16.5	+21	+10	+6	+8
Maltose:										
Uninoculated.....	6.9	6.8	6.8	7.0	6.6	+8.5	+9	+9	+11	+12
Inoculated 21 days..	8.2	8.0	7.4	8.4	7.4	+4	+16	+7	−5	+8
Lactose:										
Uninoculated.....	6.8	6.8	6.8	7.0	6.6	+9.5	+9	+9	+10	+12
Inoculated 21 days..	8.2	7.6	7.4	8.6	8.0	+8	+18	+9	−5	+8
Glycerine:										
Uninoculated.....	6.9	7.0	7.0	7.0	6.8	+8.0	+8	+9	+11	+14
Inoculated 21 days..	8.1	7.6	7.4	7.6	7.4	+10.5	+15	+9	+3	+9
Peptone water:										
Uninoculated.....	7.0	No test.	7.0	7.0	6.8	+9.0	No test.	+9	+9	+12
Inoculated 21 days..	8.1	...do ...	7.4	8.4	7.4	+8	...do ...	+7	−5	+8

SENSITIVENESS TO ACIDS AND ALKALIES

In uncorrected beef juice (titrating +23) part made alkaline with sodium hydroxid and part acidulated with hydrochloric acid the titration range of the organism is −6 to +28 (P_H 8.7 to 5.7).

Table I shows there is a reduction in the H-ion concentration after the organism has grown on the media 21 days when both Witte's and Difco peptone are used. A reduction of acid is indicated on Fuller's scale when Difco peptone is used, while with the Witte's peptone there is no uniformity of reduction or increase of acidity (indicated on Fuller's scale) though the table shows there are more cases of increase than decrease.

The Lewis organism increased in acidity from 4 to 8 points on Fuller's scale as Table II shows.

TABLE II.—*Acidity of Lewis's geranium leafspot organism*

Medium.		Fuller's scale.	Increase in acidity.
Saccharose	Uninoculated	+10	8
	Inoculated 21 days	+18	
Dextrose	Uninoculated	+10	7
	Inoculated 21 days	+17	
Maltose	Uninoculated	+8	4
	Inoculated 21 days	+12	
Lactose	Uninoculated	+8	8
	Inoculated 21 days	+16	
Glycerine	Uninoculated	+10	6
	Inoculated 21 days	+16	

In neutral beef bouillon acidulated to +22 with hydrochloric acid there is no growth. This test was made because it was not stated in Mr. Lewis's article what his foundation beef bouillon was. In the same neutral bouillon acidulated with malic acid there is growth at +10 (P_H 7.1); none at +19.5 (P_H 6.5). Acidulated with lactic acid, there is growth at +9 (P_H 7.1). There is none at +22 (P_H 6.1).

Thinking that perhaps Mr. Lewis had used beef extract and Witte's peptone instead of beef infusion and Difco peptone, tests were made using beef extract media with these acids and with tartaric acid added to the list.

There was growth in the extract (uncorrected +7) made neutral and then acidulated with malic acid to +10 (P_H 6.2); no growth in +21 (P_H 4.9).

With lactic acid there was growth in +9 (P_H 6.4); none in +18 (P_H 4.9).

With tartaric acid there was growth in +9 (P_H 6.4); none in +20 (P_H 4.7).

With hydrochloric acid there was no growth at +21 (P_H 4.0).

In peptonized beef juice made alkaline with sodium hydroxid, growth takes place to −6 (P_H 8.7). No growth takes place at −9 (P_H 9.1).

With peptonized extract of beef, the titration of which was +7 reduced with sodium hydroxid to −2, −6.5, and −13.5, there was growth at −2 and −6.5 (P_H 8.4 and 9.4, respectively).

In a long series of tests with peptone-beef bouillon in which the beef juice was used, the titration range of the organism was found to be −6 to +28 and the P_H range 8.7 to 5.7.

Mr. Lewis's notes are as follows regarding sensitiveness to acids and alkalies:

> The organism grows best in culture media that are acidulated to +15 or +20 with HCl. Growth does not occur at +40 and is retarded above +30. The same degree of acidity is not tolerated when malic, tartaric or lactic acids are employed. The growth in +8.5 lactic acid appears to be about the optimum for this acid, and the maximum is lower than for hydrochloric. Growth fails at +30 tartaric and malic.
>
> In media titrated to −10 with normal NaOH the growth is retarded, while no growth occurs at −20. In neutral bouillon growth proceeds more slowly than in +10 or +20.

MORPHOLOGY OF THE ORGANISM

The organism was studied in the cells of the spots on the leaves which had been sectioned and stained with carbol fuchsin and in 2-day-old beef agar cultures stained with carbol fuchsin. It is a short rod with rounded ends; stained in diseased material it is 0.62 μ to 1.46 μ long and 0.61 μ to 0.83 μ wide. In the agar cultures the size is 0.62 to 1.25 μ long and 0.41 μ to 1.04 μ wide. In cultures the rods hang together in chains of 2 to 16 elements, but mostly 6 to 8. Capsules were demonstrated in 2-day-old agar cultures stained with Ribbert's capsule stain and also with carbol fuchsin.

Flagella were stained with Casares-Gil's flagella stain. There is one flagellum at a pole, rarely one at each pole. One case was observed of a branched flagellum at a single pole. No spores were demonstrated in a culture of any age nor were any involution forms seen. The organism is not acid fast nor does it stain by Gram's method but is stained readily with ordinary basic aniline stains.

The morphological differences between the two organisms are slight. The size of the Lewis organism is 1.2 to 1.8 μ by 0.6 to 0.8 μ, with rare involution forms. No spores or capsules are demonstrated. It is Gram negative, not acid fast, and bears 1 to 3 polar flagella at one pole only.

NATURAL INFECTION AND CONTROL

The disease under the writer's observations is one that occurs throughout the Eastern States in greenhouses and attacks the plants usually when they are rooted cuttings and growth is being forced. We have never noticed the disease on old, slow-growing plants except in a few cases when the plant had been cured of the disease and then the spots were few and on leaves that had held over from the early attack. When the disease occurs out of doors, as it sometimes does, it is due to crowding or to unfavorable weather conditions acting on susceptible varieties. The occurrence of the disease on the grounds of the United States Department of Agriculture was mentioned in the early part of this paper. It is probable that the disease began in the greenhouse unobserved and continued when the plants were set out in the beds, hot weather and moist conditions favoring a rapid development of the disease. It has not occurred in the grounds for the last 10 years, the very susceptible varieties having been discarded.

There has never been any insect present on any of the diseased material received from the various sources. Occasionally geranium leaves are received which are infested with red spider (*Tetranychus telarius* L.), but the spotting due to this mite is of a different type.

From all the evidence gathered the organism seems to be one harbored in the soil. The disease is not a serious one unless the physical condition of the plant is weakened by too rapid growth and too moist or too warm an atmosphere with little chance of air circulating between plants, and last, but not least, too little care in watering.

Experiments leading to the control of the disease could not be undertaken in the greenhouses where the disease occurred because of distance. Control work, however, was attempted with our own plants on which the disease had been produced. The spotted leaves were picked off, the plants separated from each other in order to give them light and

plenty of air, and care was exercised in watering them. Under these conditions the disease disappeared entirely by the end of six weeks and did not return.

TECHNICAL DESCRIPTION OF THE ORGANISM

Bacterium pelargoni, n. sp.

A motile rod with rounded ends, usually borne in pairs, occurring also in chains average size 1.02 by 0.67 μ; one polar flagellum; capsules; no spores or involution forms noted; agar colonies cream colored, round, shining, with delicate internal markings; liquefies gelatin slowly; reduces litmus and methylene blue and greens the latter; produces ammonia, indol (slight) and hydrogen sulphid; does not reduce nitrates to nitrites; grows weakly in Uschinsky's and Fermi's solutions and not at all in Cohn's; coagulates sterile milk; is aerobic; has feeble diastasic action on potato starch; maximum temperature 35° C., minimum 1°, optimum 27°; thermal death point between 51° and 51.5°; resists drying six days when 6-day-old cultures are used; tolerates sodium hydroxid in peptone-beef infusion to −6 Fuller's scale (colorimetric determination P_H 8.7) and hydrochloric acid to +28 (P_H 5.7); does not produce gas from sugars or alcohols tested; is Gram negative; not acid fast; stains readily with carbol fuchsin, methylene blue, and gentian violet; is pathogenic to cultivated geraniums, causing dead spots on the leaves.

The organism is unlike the one described by Lewis from Texas as the cause of a spot disease of Erodium amd Pelargonium and may be called *Bacterium pelargoni*. It has been reported from various parts of the Eastern United States from Virginia to Massachusetts and has been under observation in the Laboratory of Plant Pathology, Bureau of Plant Industry, United States Department of Agriculture, at different times for the last 20 years.

SUMMARY

A bacterial leafspot disease of the cultivated geranium occurs widespread in the Eastern States. It is mostly a greenhouse disease but occurs occasionally on plants grown out of doors.

The organism was isolated from diseased plants received from different sources and the disease reproduced on the leaves of healthy plants.

Warm, moist conditions with poor ventilation are necessary for the organism to infect the leaves extensively.

Care in regulating the temperature, air, and moisture conditions of the greenhouse and in giving plenty of space to plants grown out of doors will go far toward preventing the appearance of the disease and toward curing it when it is present. All spotted leaves should be removed and destroyed. Very sensitive varieties should be discarded.

The name *Bacterium pelargoni* is suggested for the organism causing the disease.

PLATE 1

A.—Bacterial spots occurring on plants in Department of Agriculture grounds, Washington, D. C.

B—Bacterial spots occurring on greenhouse plants in Maryland. Natural size.


A
B

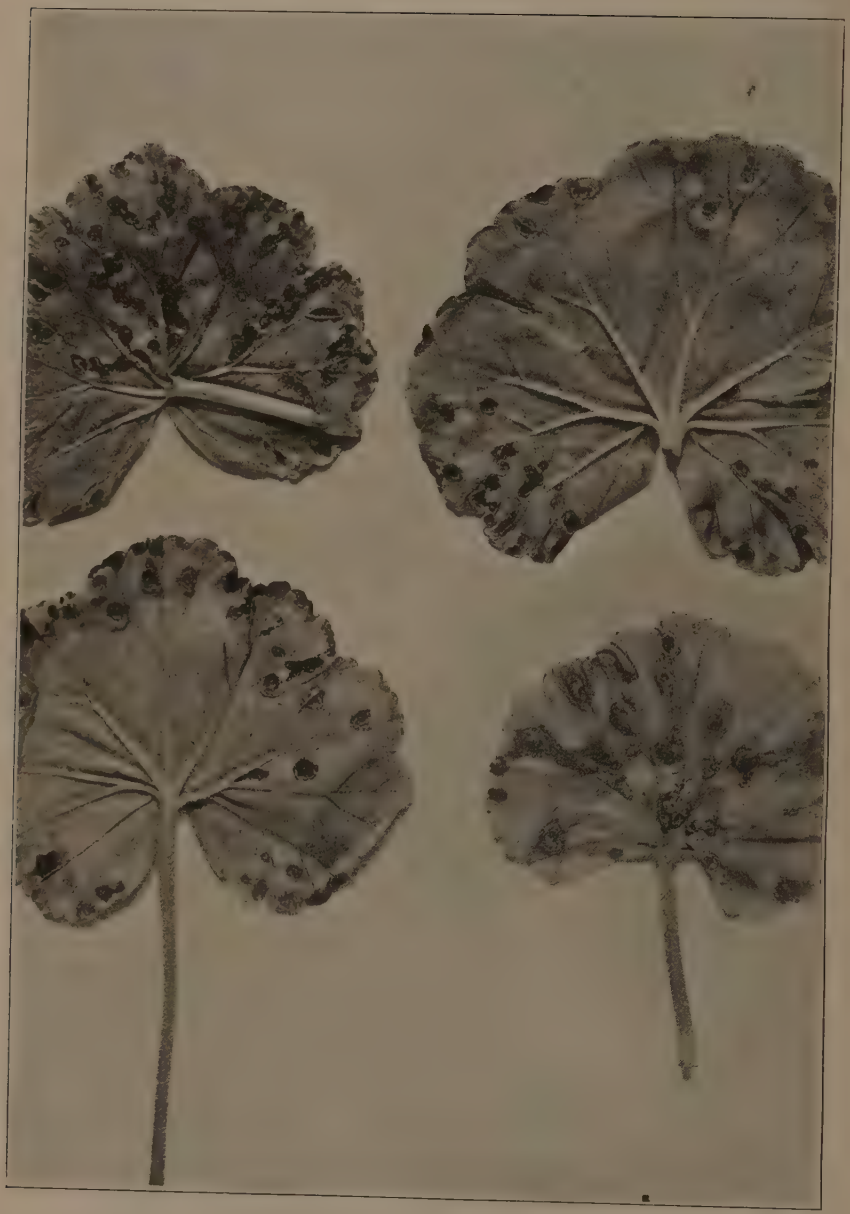


PLATE 2

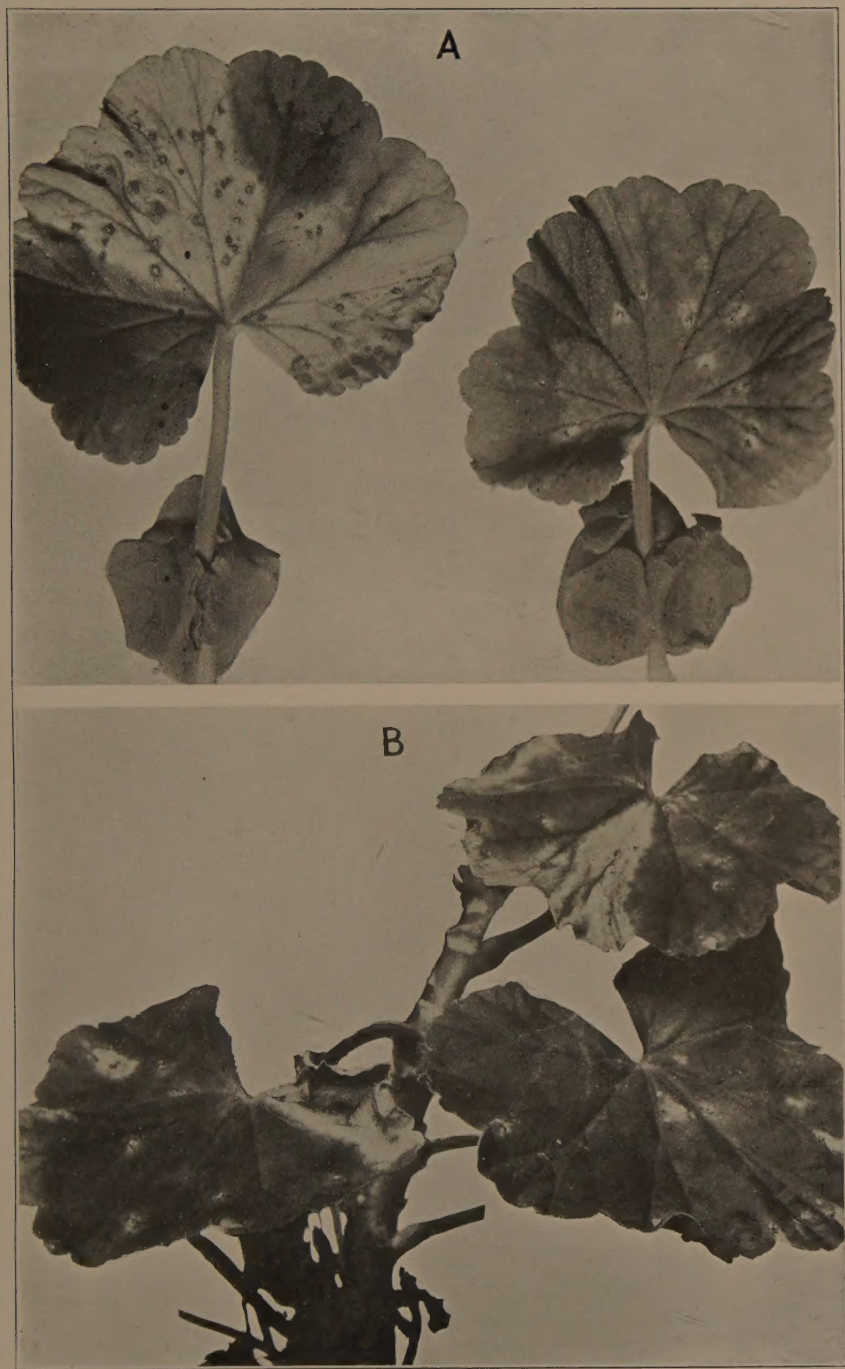
Geranium leafspot from New Jersey. Natural size.

PLATE 3

A.—Geranium leaves inoculated with leafspot organism (New Jersey) by spraying. May 5, 1920. Photographed May 26, 1920.

B.—Geranium leaves sprayed with water suspension of geranium leafspot organism (Maryland) March 31, 1915. Photographed April 25, 1915.

Natural size.


A
B